Voluntary intake and *in vivo* digestibility of forages from semi-natural grasslands in dairy cows

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Abstract

To study *in vivo* digestibility of forages from semi-natural grasslands two experiments were carried out. In the first experiment lactating dairy cows were offered three different silage-based diets. Silage originated from intensively managed grassland (IM), extensively managed species-poor grassland (SPP), or extensively managed species-rich grassland (SPR). In the second experiment lactating dairy cows were offered IM or a diet in which part of the IM had been replaced by 20% SPP (20SPP), 60% SPP (60SPP) or 60% SPR (60SPR). Intake was significantly lowest on diets with SPP, but intake on diets with SPR was not significantly lower than intake on IM. In both experiments gross energy and *in vivo* digestibility of organic matter, crude protein and neutral detergent fibre were highest for IM. In the first experiment SPP had a significantly higher digestibility than SPR, but in the second experiment differences in digestibility between 60SPP and 60SPR were not statistically significant. In both experiments *in vivo* digestibility was almost similar to *in vitro* digestibility, but no suitable equation could be found to estimate *in vitro* or *in vivo* digestibility from the chemical composition. Although digestibility and crude protein content were significantly lower for SPR than for SPP, intake of digestible organic matter appeared to be higher. It was concluded that there appears to be more scope for silage from extensively managed species-rich than for silage from extensively managed species-poor grassland.

Additional keywords: grass intake, grass species, biodiversity

Introduction

In temperate regions most grassland that is used to produce roughage for dairy cows is intensively managed, i.e., the grasslands are monocultures – mainly consisting of *Lolium perenne* – are fertilized heavily and are harvested in an early stage of maturity. During the last decades, interest in other, semi-natural grasslands with a high biodiversity has increased (Korevaar, 1986). Semi-natural grasslands have a botanical composition with more, and more diverse indigenous species, fertilization is restricted, and often the first harvesting date is delayed until the reproduction season of certain plant and bird species is over (Korevaar, 1986).

Because of their different management and the variety of forage species, semi-natural grasslands complicate the estimation of nutritional value and intake of forages. *In vivo* digestibility partly indicates the nutritional value and can be predicted from chemical composition, from *in vitro* digestibility or near infrared reflectance spectroscopy. But these methods are mainly based on calibrated data from *in vivo* trials with sheep fed *L. perenne* at maintenance level (e.g. Van Es, 1975; Steg *et al.*, 1990). Unless suitable *in vivo* standards are used to estimate *in vivo* digestibility from *in vitro* digestibility over a wide range of digestibility percentages, this indirect method does not seem appropriate for estimating *in vivo* digestibility of forages from semi-natural grasslands in lactating dairy cows. Compared with forage from intensively managed grasslands, *in vivo* digestibility of forages from semi-natural grasslands is lower (e.g. Tallowin & Jefferson, 1999), because the different genetic make-up and late harvesting will cause high contents of lignified cell wall material (Bruinenberg *et al.*, 2002). Moreover, because of their relatively low digestibility, intake of these forages is expected to be lower too (Korevaar & Van Der Wel, 1997).

Some research on *in vivo* digestibility and on intake of forages from semi-natural grasslands has been reported in literature, but the trials were mainly carried out with sheep (Armstrong *et al.*, 1986, 1989; Derrick *et al.*, 1993). As sheep are fed at a lower feeding level, it is difficult to extrapolate the results to lactating dairy cows.

In this paper we address the following subjects:

1. The effect of forages from semi-natural grassland in the diet of lactating dairy cows on voluntary intake and *in vivo* digestibility.
2. The use of indirect methods, i.e., *in vitro* digestibility and an equation based on chemical composition, to estimate *in vivo* digestibility.
3. The use of digestible nutrients of feeds to estimate their energy value.

Our study comprised two experiments. In the first experiment voluntary intake and *in vivo* digestibility of silage from two types of semi-natural grasslands were determined in lactating dairy cows. *In vivo* digestibility was compared with *in vitro* digestibility (standardized for sheep digestibility) and with the chemical composition of the silage. To be able to compare the results of silage from semi-natural grassland with silage from production grass, intensively managed grassland containing mainly *L. perenne* was included in the experiment.

In the second experiment the basal diet consisted of *L. perenne* silage. In the other diets this silage was replaced in different proportions by silage from semi-natural grassland. Because of the high fibre and low nitrogen (N) content of the silage, the

diets of dairy cows that included this silage were expected to increase rumen retention time, stimulate rumination and reduce the N surplus in the rumen. This could result in improved *in vivo* digestibility and N utilization, especially if the grass from semi-natural grassland is fed in combination with silage from intensively managed grassland. Therefore, in addition to *in vivo* digestibility, also the N balance of the second experiment is presented. Furthermore, a comparison between measured *in vivo* digestibility in Experiment 1 (feeding unmixed silage from different origins) and Experiment 2 (feeding mixtures of silage from intensively managed and semi-natural grassland) could indicate whether digestibility of the different forages is additive.

Materials and methods

Experiment 1

In Experiment 1, nine multiparous lactating dairy cows were used, weighing 585 ± 41 kg and producing – at the start of the experiment – an average of 27.6 kg milk (22.9–31.0) per day. Animals were housed in tied stalls and had free access to water. The experimental design was a 3 x 3 Latin square with 3 diets and 3 experimental periods. Each period lasted 4 weeks: the first 2 weeks for adaptation to the diet, the third week for measuring voluntary intake and the fourth week for assessing total tract digestibility at a restricted dry matter (DM[1]) intake. Three cows were used per treatment. Daily diets consisted of silage supplemented with 4 kg protein-rich concentrates and 0.4 kg additional concentrates offered in the milking parlour (for chemical composition of the concentrates see Table 1). Three types of silage were used: silage from intensively managed grassland (IM; cut 5 May 2000), silage from extensively managed species-poor grassland (SPP; cut 7 June 2000) and silage from species-rich grassland (SPR; cut 21 June 2000). The forage was pre-wilted and ensiled in big bales. For detailed information about the botanical composition of the types of silage see Bruinenberg (2003). During the first three weeks silage was offered *ad libitum*, but in the fourth week the daily amount of silage fed was restricted to 12.5 kg DM d^{-1} to prevent differences in digestibility caused by differences in DM intake.

During the whole experiment, the silage offered was weighed and sampled daily before feeding; in weeks 3 and 4 of each experimental period feed refusals were weighed and sampled daily. To measure voluntary intake, feed intake was recorded during 7 days (Saturday to Friday), and to measure digestibility, feed intake was recorded during 72 hours (Monday to Thursday). The faeces were collected quantitatively during 72 hours (Tuesday to Friday). Faeces were stored and covered immediately after excretion and weighed and proportionally sampled daily. Faecal samples were stored at –18 °C until analysis. The daily samples were combined before analysis. The protein-rich concentrates and the concentrates offered in the milking parlour were sampled once during the experiment. These samples were also stored at –18 °C until analysis.

[1] For the abbreviations used in this paper see Appendix.

Table 1. Chemical composition, gross energy (GE) and *in vitro* organic matter digestibility (d_{OM}) of the silages[1] and concentrates[2] used in the experiments. Averages over experiments and treatments.

	IM	SPP	SPR	SED[3]	Conc 1	Conc 2
Nutrient[4] (g per kg DM)						
Ash	114a[5]	94b	95b	1.8	93	76
OM	886b	906a	905a	1.8	907	924
CP	190a	132b	101c	2.5	247	176
NDF	527c	624a	563b	9.3	311	300
CF	284b	325a	316a	11.8	93	113
CFAT[6]	37a	22c	25b	0.8	49	49
NFE	375c	427b	463a	11.0	518	586
GE[6] (MJ per kg DM)	18.5a	18.4a	18.1b	0.1	18.6	18.6
d_{OM} (%)	75.8a	57.4b	54.5c	0.83	82.6	84.6

[1] IM = intensively managed grass; SPP = extensively managed spcies-poor grass; SPR = extensively managed species-rich grass.

[2] Conc 1 = protein-rich concentrates; Conc 2 = concentrates fed in milking parlour during milking.

[3] SED = standard error of difference between means.

[4] For abbreviations see Appendix.

[5] Averages in the same row, followed by a different letter are statistically different ($P < 0.05$).

[6] CFAT and GE were not analysed in Experiment 2. The values in this table are averages of Experiment 1. NFE in Experiment 2 was estimated using average CFAT contents in Experiment 1.

Experiment 2

In Experiment 2, four animals were used, weighing 610 ± 76 kg and producing – at the start of the experiment – an average of 26.6 kg (23.4–32.4) milk per day. Animals were housed in tied stalls and had free access to water. The experimental design was a 4 x 4 Latin square with 4 diets and 4 experimental periods. This experiment was part of another trial in which also fermentation characteristics were measured (Bruinenberg *et al.*, 2003a). Each experimental period lasted 3 weeks: the first 2 weeks for adaptation to the diet and the third week for recording total tract digestibility and urine production.

The animals were fed 4 different diets consisting of silage (restricted to 15 kg DM d^{-1}) and 4.5 kg DM of protein-rich concentrates per day. Silage and concentrates were the same as in Experiment 1 but the composition of the silage differed. The silage offered to the animals consisted of 100% IM (100IM), 80% IM + 20% SPP (20SPP), 40% IM + 60% SPP (60 SPP) or 40% IM + 60% SPR (60SPR).

The silage was sampled daily, during weighing. Feed refusals were weighed and sampled daily in the third week. Feed intake was recorded during 48 hours (Sunday to Tuesday) and faeces and urine were collected quantitatively during 48 hours (Monday

to Wednesday). Faeces were stored and covered immediately after excretion. To prevent urine from mixing with manure, cows were fitted with a bladder catheter (Barht). Urine was acidified with sulphuric acid to a pH between 2 and 3. Urine and faeces were proportionally sampled daily and samples were stored at –18 °C until analysis. Before analysis the daily urine samples were combined.

Laboratory analyses

The silage and faeces from Experiment 1 were air-dried at 70 °C; from Experiment 2 they were freeze-dried. The protein-rich concentrates used in both experiments were freeze-dried. Silage, concentrates and faeces were analysed for DM, ash, neutral detergent fibre (NDF) and organic N (Kjeldahl) as described by Van Vuuren *et al.* (1993) and expressed in g per kg DM. *In vitro* organic matter digestibility (d_{OM}) of silage and concentrates was determined using a modification of the method of Tilley & Terry (1963) (Van Der Meer, 1986) and expressed as percentages. Crude fat (CFAT) and crude fibre (CF) in silage, concentrates and faeces from Experiment 1 were determined according to Van Vuuren *et al.* (1991) and Tamminga (1981), and expressed in g per kg DM. Gross energy (GE, in MJ per kg DM) was determined using a bomb calorimeter (NEN-ISO 1928). In Experiment 2, indigestible acid detergent fibre (IADF) in silage, concentrates and faeces was determined according to Penning & Johnson (1983) and expressed in g kg^{-1}. Urine was analysed for organic N (Kjeldahl). DM and ash of the feed refusals in both experiments were determined as described by Van Vuuren *et al.* (1993).

Calculations

Organic matter (OM) in feed and faeces was calculated as 1000 – ash, and crude protein concentration (CP) was calculated as 6.25 × organic N. Nitrogen-free extract (NFE) was calculated as 1000 – (ash + CP + CF + CFAT).

The digestible OM (DOM) was calculated based on the *in vitro* d_{OM} and the OM content of the silage or on the chemical composition, according to the following equation (Anon., 2001a):

$$DOM = 1027 - (0.77CF + 1.23ASH + 0.03DM + 0.3D) \quad (1)$$

where D = number of days after 1 April; all other variables are in g kg^{-1}.

The metabolizable energy value of grass forages (ME, MJ kg^{-1}) was calculated based on one of the following equations (Van Es, 1978; Anon., 2001a, b):

$$ME^{1} = 15.9DCP + 37.66DFAT + 13.81DCF + 14.64DNFE - 0.63SU \quad (2)$$

$$ME^{2a} = 14.2DOM + 5.9DCP \quad (3)$$

$$ME^{2b} = 15.1DOM \quad (4)$$

where
DCP = digestible crude protein,
DFAT = digestible crude fat,
DCF = digestible crude fibre,
DNFE = digestible N-free extract,
SU = sugars (only used if > 80 g kg^{-1}), and
DOM = digestible organic matter.
All variables are in g kg^{-1}.

The N balance in Experiment 2 was calculated as total N intake minus N in milk, urine and faeces. The remaining N was called unrecovered N. In Experiment 1 the unrecovered N also includes the N in urine, as the balance was calculated as N intake minus N in milk and faeces.

Results were statistically analysed with the Analysis of Variance (ANOVA) procedure for a Latin square design, using Genstat 5 (Payne *et al.*, 1993). Treatment means were compared with Student's t-test and statistical significance was declared at $P < 0.05$.

Results

Forage analyses

Crude protein content was highest for IM and lowest for SPR. NDF content was highest for SPP and lowest for IM (Table 1). *In vitro* digestibility of OM was highest for IM and lowest for SPR. SPP and SPR were similar in composition except for CP (for SPR much lower than for SPP), NDF (for SPR much lower than for SPP) and NFE (for SPR much higher than for SPP). DOM estimated from the chemical composition was not similar to DOM calculated from *in vitro* d_{OM} (Figure 1).

Experiment 1

Voluntary intake was significantly lower for animals fed on SPP than for animals fed on SPR (Table 2), but when feeding was restricted DM intake was lowest for SPP (Table 3). OM intake was highest for SPR and IM, and CP intake was highest for IM. CP intake was lowest and the NFE intake highest for SPR.

In vivo digestibility of the different nutrients was highest for IM and lowest for SPR, except for NFE, where *in vivo* digestibility was higher for SPR than for SPP (Table 4). *In vitro* digestibility of the diets was calculated from *in vitro* digestibility of the silage and concentrates and the proportions of these different feed components in the diets. *In vivo* d_{OM} of the total diet was in accordance with the calculated *in vitro* d_{OM} of the total diet (Figure 2).

Furthermore, N efficiency (% of ingested N recovered in milk) was highest for animals fed on SPR (Table 5). The percentage of N recovered in faeces was significant-

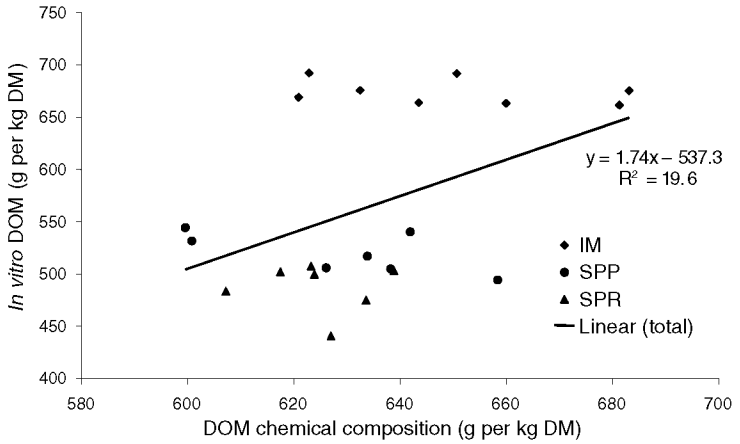

Figure 1. The relationship between estimated digestible organic matter (DOM) based on chemical composition and measured DOM based on *in vitro* digestibility of silages. See text for explanation of treatments.

ly higher for SPR than for SPP, the latter of which in turn was significantly higher than for IM.

Experiment 2

DM and OM intake were highest for animals fed on 100IM and 20SPP and lowest for animals fed on 60SPP, although 60SPR was not significantly different from either

Table 2. Voluntary intake of dry matter and nutrients from intensively managed grass (IM), species-poor grass (SPP) and species-rich grass (SPR) by dairy cows in Experiment 1 fed a fixed amount of concentrates.

	IM	SPP	SPR	SED[1]
DM intake (kg d^{-1})				
Silage *ad libitum*	13.0ab[2]	12.0b	13.2a	0.57
Concentrates	4.0	4.0	4.0	0
Concentrates in milking parlour	0.4	0.4	0.4	0
Nutrient intake[3] (kg d^{-1})				
OM	15.6ab	14.9b	16.1a	0.51
CP	3.6a	2.7b	2.5c	0.10
NDF	8.3	9.0	8.7	0.33

[1] SED = standard error of the difference between means.

[2] Averages in the same row, followed by a different letter are statistically different ($P < 0.05$).

[3] For abbreviations see Appendix.

Table 3. Dry matter intake, nutrient intake and gross energy (GE) in the digestibility study of Experiments 1 and 2 (including concentrates). For the experimental treatments see Materials and methods.

	Experiment 1				Experiment 2				
	IM	SPP	SPR	SED[1]	100IM	20SPP	60SPP	60SPR	SED
DM intake (kg d^{-1})									
Silage	12.0a[2]	10.4b	11.6a	0.29	13.9a	13.6a	12.2b	12.8ab	0.44
Concentrate 1	4.0	4.0	4.0	0	4.5	4.5	4.5	4.5	0
Concentrate 2[3]	0.29	0.29	0.29	0	–[4]	–	–	–	–
Total intake	16.3a	14.7b	16.0a	0.29	18.3a	18.1a	16.7b	17.3ab	0.40
Nutrient[5] *intake* (kg d^{-1})									
OM	14.6a	13.4b	14.4a	0.26	16.3a	16.1a	15.0b	15.5ab	0.39
CP	3.4a	2.5b	2.3c	0.04	3.7a	3.6b	3.0c	2.9c	0.08
NDF	7.8	7.9	7.9	0.22	8.6	8.7	8.3	8.4	0.30
CF	4.1	4.0	4.1	0.10	4.0	4.0	3.8	4.1	0.16
CFAT	0.67a	0.46c	0.52b	0.01	–	–	–	–	–
NFE	6.4b	6.5b	7.5a	0.13	7.8	7.9	7.6	7.9	0.21
IADF	–	–	–	–	0.71c	0.82c	0.99b	1.35a	0.063
GE (MJ d^{-1})	303a	274b	292a	5.5	–	–	–	–	–

[1] SED = standard error of the difference between means.

[2] Averages in the same row and within the same experiment, followed by different letters are statistically different ($P < 0.05$).

[3] Concentrates fed in the milking parlour during milking. Not fed in Experiment 2.

[4] – = not measured.

[5] For abbreviations see Appendix.

(Table 3). Crude protein intake was highest for 100IM and lowest for 60SPP and 60SPR. Intake of IADF was highest for 60SPR and lowest for 100IM and 20SPP. No differences were observed in NDF and CF intake.

The *in vivo* digestibilities of OM (d_{OM}), DM (d_{DM}), CP (d_{CP}), NDF (d_{NDF}) and IAFD (d_{IADF}) in the total diet were all highest for 100IM and 20SPP and lowest for 60SPR (Table 4). The *in vivo* digestibility was also significantly lower for 60SPP than for 100IM and 20SPP, for all nutrients, except for IADF, as d_{IADF} was not significantly lower on 60SPP compared with 100IM. For 20SPP and 100IM, *in vivo* d_{OM} and d_{NDF} were significantly different, but not *in vivo* d_{DM}, d_{CP} and d_{IADF}. *In vivo* d_{OM} of the total diet was in accordance with the calculated *in vitro* d_{OM} of the total diet (Figure 2).

Also in Experiment 2 the diet that contained SPR had the highest N efficiency and 100IM the lowest (Table 5), but not all differences were statistically significant. The highest N concentration in urine was observed for animals fed on 60SPP (Table 5). The percentage N excreted in faeces was highest for 60SPR (Table 5).

Table 4. *In vivo* nutrient digestibility coefficients (%) in Experiments 1 and 2. For the experimental treatments see Materials and methods.

Nutrient[1]	Experiment 1				Experiment 2				
	IM	SPP	SPR	SED[2]	100IM	20SPP	60SPP	60SPR	SED
DM	76.8a[3]	64.0b	61.2c	1.01	75.8a	73.8a	68.9b	67.6b	0.84
ASH	59.3a	46.9b	38.7c	1.47	61.3a	59.6a	55.3b	51.5c	1.02
OM	78.9a	65.7b	63.5c	0.97	77.6a	75.5b	70.4c	69.4c	0.82
CP	71.1a	61.6b	55.5c	1.16	70.8a	70.2a	67.1b	64.5c	0.78
NDF	81.8a	62.6b	55.6c	1.19	84.8b	81.4b	73.8c	72.3c	1.07
CF	83.4a	63.7b	54.6c	1.14	–[4]	–	–	–	–
NFE	80.4a	67.6c	70.3b	1.12	–	–	–	–	–
IADF	–	–	–	–	25.6ab	27.9a	22.2bc	19.9c	2.26
GE	75.8a	63.0b	60.4c	1.09	–	–	–	–	–

[1] For abbreviations see Appendix.

[2] SED = standard error of the difference between means.

[3] Averages in the same row and within the same experiment, followed by different letters are statistically different ($P < 0.05$).

[4] – = not measured. Apart from CF and GE, CFAT in Experiment 2 was not measured either. As a result neither NFE could be calculated.

Table 5. Intake and excretion of N in Experiments 1 and 2. The amount of N excreted is expressed as a percentage of N intake.

	Experiment 1				Experiment 2				
	IM	SPP	SPR	SED[1]	100IM	20SPP	60SPP	60SPR	SED
N intake (g d^{-1})	546a[2]	396b	365c	6.3	604a	566a	481b	465b	13.9
N excreted (%)									
Milk	20b	21b	24a	0.9	18c	19bc	20b	22a	0.4
Faeces	29c	38b	44a	1.2	29c	31c	33b	36a	0.6
Urine	–[3]	–	–	–	41	42	43	36	3.4
Unrecovered[4]	52a	40b	32c	1.5	12	8	5	7	3.1

[1] SED = standard error of the difference between means.

[2] Averages in the same row and within the same experiment, followed by different letters are statistically different ($P < 0.05$).

[3] – = not measured.

[4] The unrecovered N in Experiment 1 is the ingested N not excreted via the milk and faeces. In Experiment 2 the unrecovered N is the ingested N not excreted via the milk, faeces and urine.

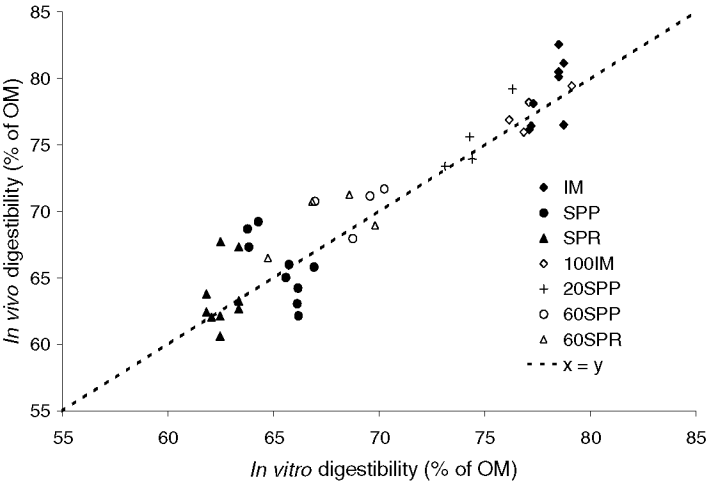

Figure 2. The relationship between *in vitro* and *in vivo* organic matter (OM) digestibility of the diets used in the experiments. See text for explanation of treatments.

Discussion

Intake

The low voluntary intake of DM for animals fed on SPP and 60SPP is in accordance with the results from another experiment (Bruinenberg, 2003) and was probably caused by the high NDF content (Table 1) and the low NDF degradability of SPP (Bruinenberg *et al.*, 2003a). A high NDF content in the diet is expected to increase resistance to physical breakdown and rumen fill, resulting in a lower voluntary intake (Armstrong *et al.*, 1986; De Visser *et al.*, 1998). This is confirmed by the relatively high DM intake for animals fed on SPR, which compared with SPP had a relatively low NDF content. The high NDF intake for 100IM and 20SPP might seem contrary to a limitation of NDF for intake, but the degradation rate of NDF on 100IM was higher than for SPP or SPR (Bruinenberg *et al.*, 2003b). No relationships were observed between other chemical characteristics or digestibility of the silages and voluntary intake.

Digestibility

Factors affecting the difference in *in vivo* and *in vitro* digestibility between IM and SPP or SPR have been discussed by Bruinenberg *et al.* (2002). In short, these factors include differences in stage of maturity, in forage species and in anatomical structure between forage species. In this discussion we will focus on the different indirect methods to estimate the *in vivo* digestibility in dairy cows and the possibility to use these methods for SPP and SPR.

The lack of differences between the *in vivo* and the *in vitro* digestibility suggests that the *in vitro* method estimates the *in vivo* digestibility of the forages used in this study well. This was not expected. The *in vitro* digestibility is standardized for wethers fed at maintenance level (Tilley & Terry, 1963; Steg *et al.*, 1990; Anon., 2001a) and not for dairy cows at higher feeding levels, and the *in vivo* digestibility is not a standard value as it is influenced by diet and animal factors. Furthermore, differences in microbial activity could cause differences in *in vitro* digestibility. However, some standard samples with a known *in vivo* digestibility are included in the *in vitro* digestibility analysis to correct for differences in activity of the rumen fluid. Another problem when using the *in vitro* method for samples as used in this study is that standards used to correct for differences in activity of rumen fluid may not be appropriate. However, as the *in vivo* and *in vitro* digestibility had approximately similar values, there did not seem to be a problem with the standards.

Digestible OM (DOM) can also be estimated from the chemical composition of the silage (Equation 1; Anon., 2001a). In practice, Equation 1 is not used any longer because for routine analysis estimating DOM, near infrared reflectance spectroscopy is more efficient. But because of lack of good calibrations this technique is not appropriate for forages from semi-natural grasslands. So it is important to know whether DOM can be estimated from the chemical composition. It was observed that Equation 1 is not correct for any of the silages (Figure 1). It can therefore be concluded that in this study there was no relationship between DOM estimated from the chemical composition and DOM estimated from the *in vitro* digestibility. Differences were probably due to stage of maturity, botanical composition, cell wall content, or other differences among silages. Anon. (2001a) already indicated that Equation 1 should not be used for forages with a diverse botanical composition, but in our study it did not appear to be correct for the silage from intensively managed grassland either.

For semi-natural grasslands the variable *D* in Equation 1 is an important cause for an incorrect estimate. Usually *D* corrects for seasonal effects, such as temperature. However, for semi-natural grasslands the late date of first cut is probably more important than the advanced season, as a delayed first cut results in an advanced stage of maturity. Therefore the effect of *D* is underestimated in the forage from semi-natural grasslands. Stage of maturity affects degradability and digestibility (Bosch *et al.*, 1992). So a correction of Equation 1 for stage of maturity, providing for an extra reduction in digestibility if the first cut is delayed or if higher percentages of plants have elongated their stems, would probably improve the estimate of DOM.

In vivo d_{OM} of the silage was calculated from *in vivo* d_{OM} of the diet and *in vitro* digestibility of the concentrates (which was assumed to be equal to the *in vivo* digestibility of the concentrates). Also for other nutrients such calculations were made, but assumptions had to be made for the digestibility of the nutrients in the concentrates. *In vivo* d_{DM}, d_{CP} and d_{NDF} of the concentrates were assumed to be 80, 76 and 70%, respectively (based on composition and digestibility of the components in Anon., 2001b). Results of the calculations are shown in Table 6. Comparing *in vitro* d_{OM} of the silages (Table 1) with *in vivo* d_{OM} (Table 6), the latter appeared a few percentage points higher. But the differences are small, so it may be concluded that *in vitro* d_{OM} of the silage approaches the actual value reasonably well. Because *in vivo* d_{OM} of all diets was

Table 6. The nutrient digestibility[1] (d) of silage from intensively managed (IM), species-poor (SPP) and species-rich (SPR) grassland as calculated from the digestibility for the treatments in Experiments 1 and 2. Values in %.

Nutrient[2]	Experiment 1			Experiment 2			
	IM	SPP	SPR	100IM	SPP a[3]	SPP b	SPR
DM	75.6	57.4	54.2	74.4	61.1	58.5	55.6
OM	77.6	58.7	56.4	76.0	61.7	59.2	57.1
CP	69.4	55.7	47.9	69.1	64.9	60.3	54.6
NDF	86.0	59.5	50.3	89.6	67.6	65.7	61.9

[1] For example, d IM was calculated with the formula d IM = (d diet – d concentrates * % concentrates) / % IM. The digestibility of the concentrates is based on *in vitro* digestibility: d_{DM} = 80%; d_{OM} = 82.6%; d_{CP} = 76%; d_{NDF} = 70%. The neutral detergent fibre digestibility (d_{NDF}) of the concentrates is based on the composition of the concentrates (Bruinenberg *et al.*, 2003b) and the crude fibre digestibility of these components (Anon., 2001b).

[2] For abbreviations see Appendix.

[3] SPP a is based on 20SPP; SPP b is based on 60SPP (see Materials and methods).

estimated correctly using *in vitro* data – which is confirmed by Figure 2 – it can also be concluded that there was no positive nor negative effect on d_{OM} when part of the IM was replaced by SPP or SPR. In addition, from the comparisons between the different replacement percentages in the treatments it was concluded that d_{OM} was additive. *In vivo* d_{OM} of the diets 20SPP, 60SPP and 60SPR was as expected from *in vivo* d_{OM} based on the proportions by weight and d_{OM} of 100IM, SPP and SPR (Figure 3). d_{NDF} and d_{CP} were not completely linearly correlated with percentage replacement (Figure 3; NDF), which was attributed to differences between animals, as *in vivo* digestibility of SPP and SPR were measured in other animals than digestibility of 60 SPP and 60SPR.

Metabolizable energy intake

In the Netherlands, metabolizable energy (ME) of grass forages is estimated based on the Equations 2, 3 or 4 (Van Es, 1978; Anon., 2001a, b). Also in other countries ME is often calculated from the digestible nutrient contents in the feed (Van Der Honing & Alderman, 1989; Beever *et al.*, 2000).

In all cases Equations 3 and 4 gave lower predictions than Equation 2. However, comparing Equation 2 with Equations 3 and 4, total *ME* intake differed about 5 MJ d^{-1} at the most, which is equivalent to approximately 1 kg of milk per day. Except for SPR, predictions of ME^{2b} differed more from ME^{1} than ME^{2a}. So although Equation 3 gave lower values than Equation 2 for IM as well as for SPP and SPR, the discrepancy between the two remained small. So Equations 3 and 4 can be used for forages from semi-natural grasslands.

Total DOM intake was higher for animals fed on (60)SPR than on (60)SPP,

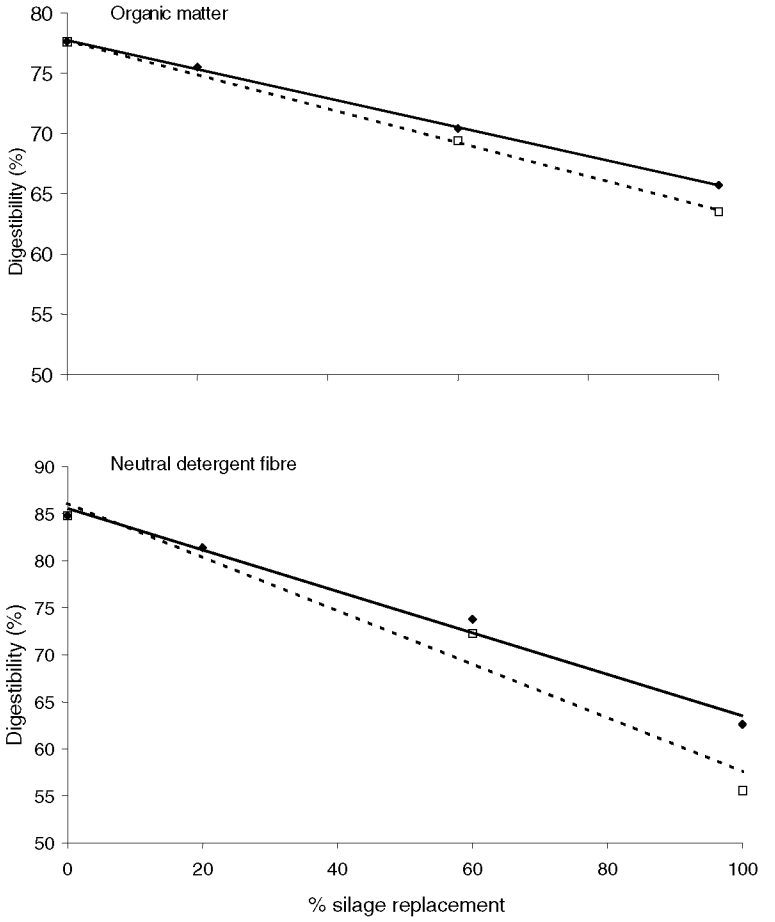

Figure 3. Organic matter and neutral detergent fibre digestibility of silage diets as affected by replacing part of the silage from intensively managed grassland by silage from species-rich (◆;solid line) or silage from species-poor grassland (□; broken line).

although the digestibility of (60)SPP was higher than of (60)SPR. This was due to the higher DM intake for (60)SPR. A higher DOM intake will also result in a higher ME intake, and thus in a higher net energy intake, resulting in a higher expected milk production. So it would be interesting to compare expected and actual milk output for the different treatments. However, because of the experimental design used in our study, differences in milk production between treatments could not be tested independently for statistical significance.

Nitrogen balance

Because of the short measuring periods, in this study the term unrecovered N is used instead of N retention, as it is unlikely that over a period of two to three days N is retained in the body. Moreover, between-days variation in N excreted via urine or milk will have occurred, and finally, it was not clear whether to allocate unrecovered N to urine or to milk.

No positive effects of mixing IM with SPP or SPR on efficiency of N utilization were observed, but also this could be due to the statistical design. The high N intake for animals fed on IM, 100IM and 20SPP resulted in a relatively low efficiency of N utilization for milk, whereas the low N intake for animals fed on SPR resulted in a relatively high efficiency of N utilization for milk, even though milk production was reduced. The low N recovery in the milk for IM, 100IM and 20SPP coincided with high recoveries in the urine or high unrecovered N, which would result in high N losses to the environment. The high proportion of N in urine for 60SPP is not considered remarkable, as the unrecovered fraction is lower than on the other diets in Experiment 2. As expected, N recoveries in urine and unrecovered N were lowest for SPR, which was attributed to the low N intake and the low CP digestion on SPR.

Conclusions

In vitro digestibility gave a good indication of *in vivo* digestibility. Moreover, when IM was combined with SPP or SPR, *in vivo* OM digestibility was additive. Our results confirm that the equation to predict DOM from the forage's chemical composition (Anon., 2001a) is not valid for silage from semi-natural grasslands.

Although (*in vivo* and *in vitro*) digestibility and CP content were higher for SPP than for SPR, in both experiments DOM intake was highest for animals fed diets with SPR because of a higher DM intake. So there may be more scope for the use of SPR than of SPP in diets of highly productive dairy cows.

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Appendix

Abbreviations used

d	digestibility
ANOVA	analysis of variance
CF	crude fibre
CFAT	crude fat
CP	crude protein
D	number of days after 1 April
DCF	digestible crude fibre
DCP	digestible crude protein
DFAT	digestible crude fat
DM	dry matter
DNDF	digestible neutral detergent fibre
DNFE	digestible nitrogen-free extract
DOM	digestible organic matter
GE	gross energy
IADF	indigestible acid detergent fibre
IM	silage from intensively managed grassland
ME	metabolizable energy
MEI	metabolizable energy intake
N	nitrogen
NDF	neutral detergent fibre
NFE	nitrogen-free extract
OM	organic matter
SPP	silage from extensively managed species-poor grassland
SPR	silage from extensively managed species-rich grassland
SU	sugars